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Innovative Approach for the Use of Huwa-San TR50 in Controlling Cotton Aphids (*Aphis gossypii* Glover)

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Abstract

Chemical control remains the main method of controlling the cotton aphid (*Aphis gossypii* Glover). Millions of dollars have been lost due to plant damage which resulted in reduced quality and yield of cotton. Nevertheless, *A. gossypii* can rapidly develop resistance to different groups of insecticides such as organophosphates, carbamates and pyrethroids. The potential of Huwa-San TR50 in controlling *A. gossypii* is yet to be tested. Huwa-San TR50 is a formula of hydrogen peroxide which has been stabilized by the addition of a small quantity of silver and has extensively used as a disinfectant. In this study, it was found to be very potent in killing *A. gossypii* and produced 93.5, 96.5, 97 and 95.5% mortality at 1000, 2000, 3000 and 4000 ppm, respectively, after 48 h of exposure. Furthermore, there was no significant difference between four Huwa-San TR50 concentrations after 48 h of exposure, on the mortality of *A. gossypii*. Huwa-San TR50 of up to 4000 ppm had no observable effects on the mortality and behavior of adult honeybee workers (*Apis mellifera lamarckii*) as compared with the control. Also, Huwa-San TR50 concentration of up to 3000 ppm had no observable effect on seven-spot ladybird beetles (*Coccinella septempunctata*) whereas a concentration of 4000 ppm produced 100% mortality after 24 h of exposure. Huwa-San TR50 concentrations of up to 2000 ppm failed to produce any symptoms on cucumber leaves. The differential effects of Huwa-San TR50 on aphids and beneficial insects, suggest the need for further investigation to understand the effects of Huwa-San TR50 on other host plants of aphids and aphid species.

Keywords: Huwa-San TR50, aphids, *Aphis gossypii* Glover, honeybees, ladybird beetles

1. Introduction

The cotton aphid (*Aphis gossypii* Glover) is a serious insect pest in horticulture, agriculture and greenhouse crops (Aheer et al., 2006; Khattak et al., 2007). This is because more than 320 suitable host plants belonging to 46 families worldwide have been reported as hosts of *A. gossypii* (Blackman & Eastop, 2000). It has also been considered as an important pest of cotton, as a result of the high economic losses of cotton recorded (Ebrt & Cartwright, 1997). In California for example, cotton infestation by *A. gossypii* in the mid- or late season, can significantly reduce both cotton yield and lint quality (Godfrey & Fuson, 2001) and is responsible for about US $ 24 million in crop loss and another US $ 38 million in control costs (Godfrey & Fuson, 2001). This pest can also infest plants at the beginning of the cropping season. Plants infested by *A. gossypii* show reduced development, especially in young leaves which are responsible for driving the main stem and/or branches (Leclant & Deguine, 1994; Ebert, 2008). This includes direct damage to the plant, honeydew on the plant leaves and open lint which can result in a decline in the quality and yield of cotton by promoting the growth of black sooty mold fungus that causes problems during spinning (Deguine et al., 2000).

In cotton fields, *A. gossypii* is primarily controlled by seed treatment or foliar spraying of insecticides (systemic or broad-spectrum) (Almeida et al., 2008; Torres & Silva-Torres, 2008). In organic farming, the biological control (predatory and parasitic insects) of *A. gossypii* is effective at low density (Kaleem et al., 2014) but can be less effective under adverse environmental conditions (Godfrey & Fuson, 2001; Kaleem et al., 2014), *e.g.* reduced populations of natural enemies (Grafton-Cardwell et al., 1997). This results in an increase in the difficulty of controlling many pests including *A. gossypii* (Pinto et al., 2013).

Insecticides remain the primary tool for growers in controlling *A. gossypii*. Nevertheless, *A. gossypii* can rapidly develop resistance to several insecticides, making the control of *A. gossypii* difficult and ineffective (Godfrey & Fuson, 2001). Previous studies have reported the resistance of *A. gossypii* to insecticides (Funk et al., 1980; Takada & Murakami, 1988; Grafton-Cardwell et al., 1992; Guburn et al., 1992). This resistance to organophosphates, carbamate and pyrethroids has developed in many parts of the world including China (Sun et al., 1987; Zheng et al., 1988), Japan (Saito, 1989; Hama et al., 1995), the United States (Grafton-Cardwell et al., 1991; Hollingsworth et al., 1994), Israel (Ishaaya & Mendelson, 1987), the United Kingdom (Funk et al., 1980; Funk et al., 1993) and Sudan (Guburn et al., 1992). In the San Joaquin Valley of California, Grafton-Cardwell et al. (1991) reported a temporal and spatial variation in susceptibility to selected organochlorine and organophosphate.

In agricultural greenhouses, insect pest management has extensively been used for both natural enemies (mainly in biological control) and insecticides. The latter has widely been used as a simple tool to control a wide range of insect pests without thinking about the serious damage they might produce in both natural enemies and beneficial insects. Furthermore, the extensive use, misuse and/or unnecessary application of insecticides at the early season might result in a reduction in the population of natural enemies as well as beneficial insects. Widespread concern has been expressed about the exposure of honeybees to insecticides by direct contact or indirect residues in nectar and/or pollen. For example, an acute risk to honeybees was identified from exposure to neonicotinoid insecticide residues in nectar and pollen or dust from seed treatment in maize, cereal and oil-seeds (European Food Safety Authority, 2013).

Ladybird beetles are very effective in controlling aphids (Seo & Youn, 2000, 2002), however, the use of insecticides such as imidacloprid and acetamiprid at the recommended field rate for aphid control has been reported to kill ladybird beetles (Youn et al., 2003). Ladybird beetles might also be indirectly exposed to insecticide residues after consuming treated aphids (De Cock et al., 1996).

Hence, many insecticides have been banned or restricted in some countries as a result of their toxicity to natural enemies and/or beneficial insects. For example, clothianidin, thiamethoxam and imidacloprid have been restricted in the EU since 2013, due to their high risk to honeybees (EU, 2013). This is because these insecticides are very effective in controlling many insect pests including aphids. These combined problems can make the control and management of *A. gossypii* very difficult.

The literature review has highlighted the need to investigate an alternative strategy for controlling *A. gossypii* in order to replace the traditional, over used, banned, or restricted insecticides.

There are no previous reports of using Huwa-San TR50 for the control of cotton aphids. Huwa-San TR50 is a formula of hydrogen peroxide which has been stabilized by the addition of a small quantity of silver (www.huwasa.com). Huwa-San TR50 is mainly used as a disinfectant and was developed over twenty years ago (www.huwasa.com). Huwa-SanTR50 has several advantages that make it reliable and safe such as long term effectiveness, high efficacy even at low concentrations, being effective under a wide range of temperatures up to boiling point, gentle to the skin, biodegradable, non-toxic, no build-up of resistance by microorganisms, colorless, odorless and tasteless (www.huwasa.com).

There is no information on the impact of the application of Huwa-San TR50 on pests or beneficial insects. This study aimed to evaluate the potential effectiveness of Huwa-San TR50 against aphids; test the side effects of Huwa-San TR50 on beneficial insect honeybees and ladybird beetles; determine the recommended rate that can be used in the field without any serious leaf malformation.

2. Method

Huwa-San TR50 was obtained from Ghatafan Company in Onaizah (retailer agent). The stock solution of Huwa-San TR50 (500,000 ppm) was diluted with distilled water to give a serial concentration range between 1000 to 4000 ppm.

Untreated cucumber leaves (*Cucumis sativus*) (Brenji RZ F1-Hybrid (22-80)) were used to maintain *A. gossypii* in greenhouses. The experiments in this study were conducted under laboratory and greenhouse conditions.

In greenhouse experiments, four different Huwa-San TR50 concentrations (1000, 2000, 3000 and 4000 ppm) including the control (only distilled water without Huwa-San TR50) were sprayed directly on the cucumber leaves (mid-stage) by using a knapsack sprayer (20 L).

In the laboratory, cucumber leaves infested by *A. gossypii* were collected from the greenhouse. The number of *A. gossypii* on each leaf was counted. Thereafter, the leaves were distributed in separate containers. Huwa-San TR50 was then sprayed directly on the infected cucumber leaves by using a hand sprayer. The mortality of *A. gossypii* was determined after 24 and 48 h by counting dead *A. gossypii* under a microscope.

Adult worker honeybees (*Apis mellifera lamarckii*) were obtained from the Agricultural Research Station of Qassim University. The four Huwa-San TR50 concentrations listed above including the control (only distilled water without Huwa-San TR50) and beeswax (as a source of food) were sprayed directly onto 25 honeybees with four replicates for each concentration. Beeswax was included with each treatment including the control (only distilled water without Huwa-San TR50). Beeswax was also exposed to Huwa-San TR50 to determine if it can produce any effect by feeding. The mortality of the honeybees was measured after 24 and 48 h and the effect on behavior was noted.

Seven-spot ladybird beetles (*C. septempunctata*) were collected from Alfalfa fields and placed in separate containers containing cucumber leaves infested by *A. gossypii,* which served as a food source. The four Huwa-San TR50 concentrations listed above including the control (only distilled water without Huwa-San TR50) were sprayed directly on ten ladybird beetles with four replicates for each concentration. The mortality of ladybird beetles was measured after 24 and 48 h and the behavior was observed.

The mortality of the cotton aphids was calculated manually under a microscope. Also, the mortalities of honeybees and ladybird beetles were calculated manually by direct observation. The collected data for all variables were statistically analyzed using the MSTATC microcomputer program (MSTATC, 1990). Data were then calculated using the Microsoft Excel program. Dilution-response curves for the mortality assay were plotted using Graph pad Prism version 7. Data points were the mean ± SEM of each concentration and the graphs were fitted using a nonlinear regression with a four parameter logistic equation where the upper plateau was set to 100% and the lower plateau was set to 0.

3. Results

A. gossypii were exposed to four Huwa-San TR50 concentrations (1000, 2000, 3000 and 4000 ppm) and showed 93.5, 96.5, 97, and 95.5% mortality, respectively, after 48 h of exposure as compared with the control. Furthermore, there was no significant difference between the mortality of *A. gossypii* caused by 48 h exposure to Huwa-San TR50 concentrations of 1000, 2000, 3000 and 4000 ppm. However, there was a significant difference in the mortality percentage between 24 and 48 h (Figure 1).

Honeybees were also very active and no difference was observed between the treated and non-treated honeybees (control), whether after 24 or 48 h of exposure at any concentrations tested. Huwa-San TR50 surprisingly had no significant effect on honeybees and ladybird beetles. No significant effect was seen on ladybird beetles up to 3000 ppm whereas at 4000 ppm, all ladybird beetles were killed. Huwa-San TR50 did not produce any symptoms on cucumber leaves up to 2000 ppm whereas 3000 ppm and beyond produced detectable leaf malformation after 48 h of exposure (Figure 2).

Table 2. Percentage mortality of *A. gossypii* after 24 and 48 h of exposure to Huwa- San TR50. Mean of four replications

Huwa-San TR50 concentration (ppm)	24 h	48 h
4000	41.5a	95.5a
3000	36.0b	97.0a
2000	42.5a	96.5a
1000	11.5c	93.5a
Control (distilled water)	1.9d	6.3b

Table 2. Percentage mortality of ladybird beetles after 24 and 48 h of exposure to Huwa-San TR50. Mean of four replications

Huwa-San TR50 concentration (ppm)	24 h	48 h
4000	100a	100a
3000	13.3b	30.0b
2000	10.0b	16.6b
1000	10.0b	16.6b
Control (distilled water)	6.6b	16.6b

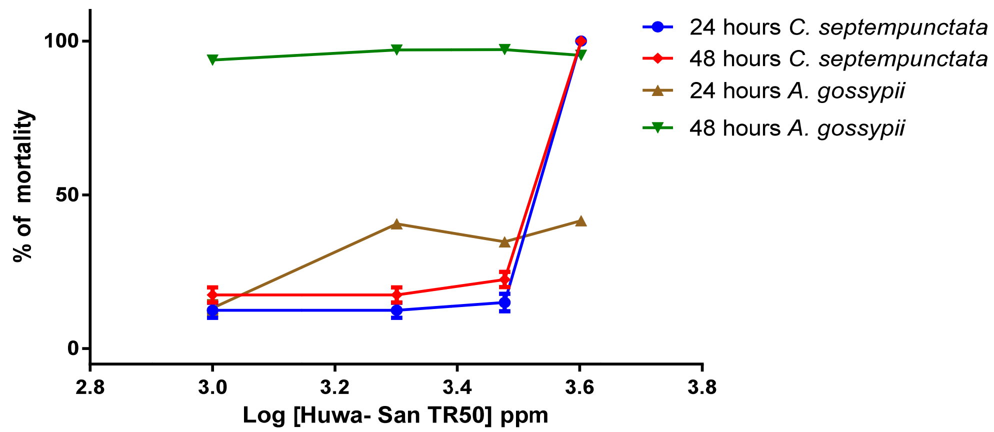
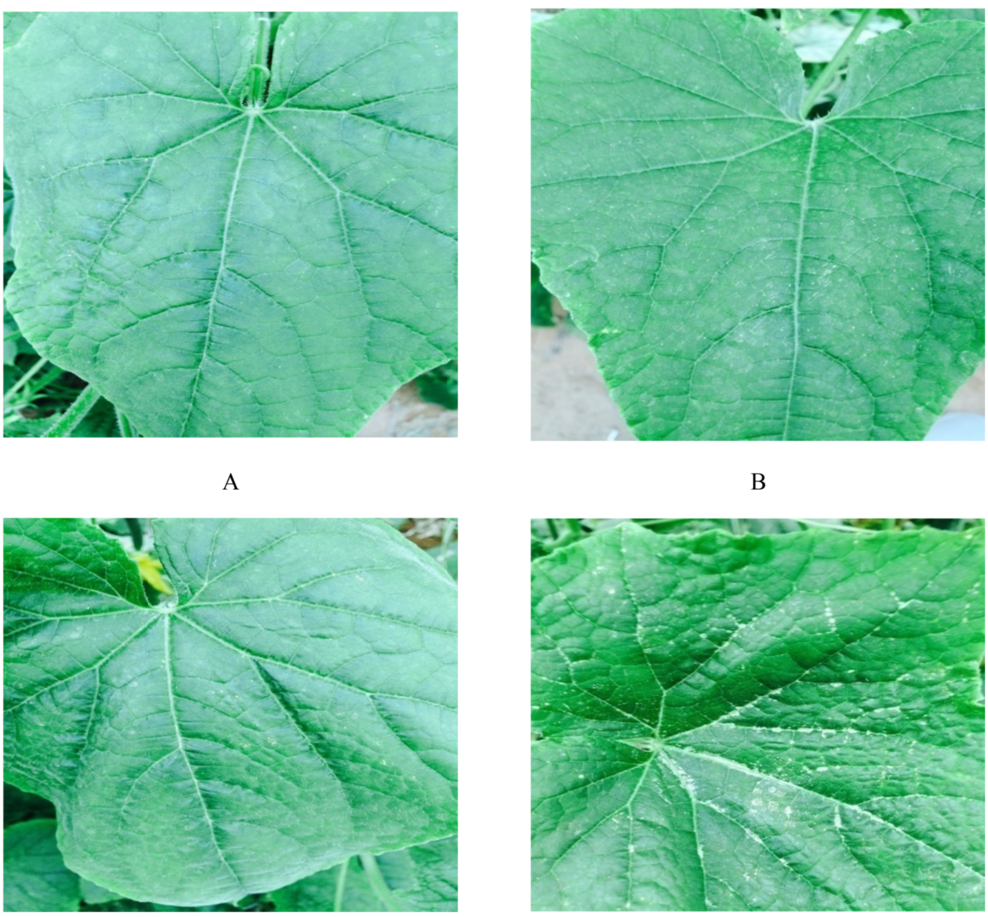


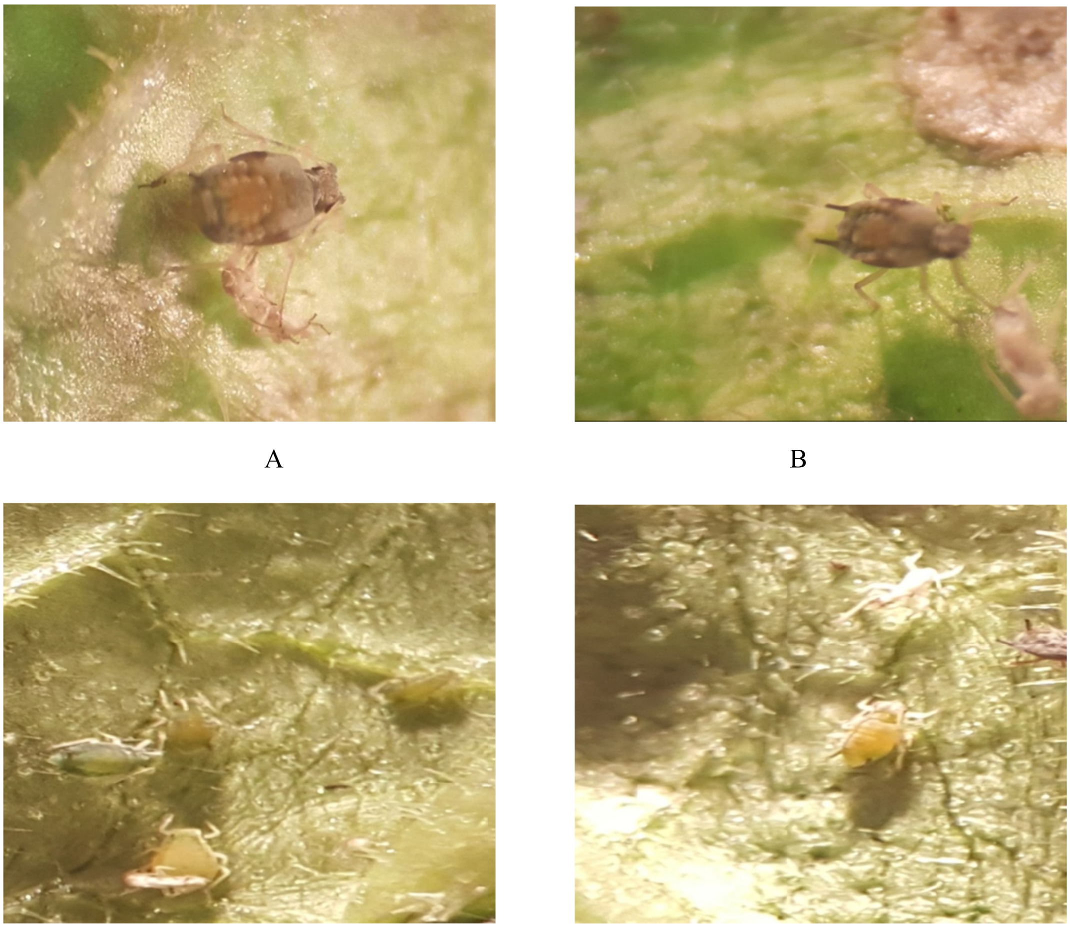
Figure 1. Comparison of the effects of Huwa-San TR50 on the mortality of *A. gossypii* and ladybird beetles (*C. septempunctata*) after 24 and 48 h of exposure, expressed as a percentage of the control mortality in distilled water. Each point is the mean ± SEM of 4 replicates, but in most cases the error bars are smaller than the symbols used. The lines were fitted using a non-linear regression in Graph Pad Prism 7 with the maximum plateau being 100% and the minimum being 0%



A B

C D

Figure 2. Pictures (A 1000 and B 2000 ppm) showing cucumber leaves with no leaf malformation and detectable leaf malformation at (C 3000 and D 4000 ppm) after the field application of Huwa-San TR50



A B

C D

Figure 3. Pictures (A) and (B) show the cuticle damage of the treated *A. gossypii* with 2000 ppm of Huwa-San TR50 as compared with non-treated *A. gossypii* (control) (C) & (D)

4. Discussion

Many chemical compounds with a range of formulations have been extensively used and sold commercially as insecticides in agriculture. The extensive use of these insecticides might increase insect resistance and affect non-target species, especially when growers exceed the recommended application rate for the control of resistant pests. Also, the excessive use of chemicals can increase insecticide residues in food and the environment. In the last three decades, several previous studies have reported resistance to insecticides in *A. gossypii* (Funk et al., 1980; Takada & Murakami, 1988; Grafton-Cardwell et al., 1992; Guburn et al., 1992).

Moores et al. (1996) investigated the resistance of two aphid clones (968E and 1081K) which were originally collected from cotton in Greece in the year 1991 and Zimbabwe in 1992, respectively. They found that the resistance ratios of 968E as compared with the wild type were > 380-fold for primicarb, 180-fold for demeton-S-methyl, 5-fold or less for methomyl and 5-fold for pirimiphos-methyl whereas the resistance ratios of 1081K were 74-fold for pirimicarb, 57-fold for triazamate and 9- to 18-fold for methomyl and pirimiphos-methyl. These resistance ratios explain the difficulty of controlling *A. gossypii*.

The environmental impacts of insecticide usage as well as the development of resistance to insecticides have encouraged us to investigate a new strategy. The results shown here with Huwa-San TR50 were unexpected, as it can effectively kill *A. gossypii* at certain concentrations whilst having much less effect on non-target/beneficial insects at the same concentrations. Huwa-San TR50 was highly effective in killing *A. gossypii* and produced mortality of 11.5, 42.5, 36.0 and 41.5% after 24 h and 93.5, 96.5, 97.0 and 95.5% after 48 h of exposure at 1000, 2000, 3000 and 4000 ppm, respectively. Minimal impacts were recorded for both honeybees and ladybird beetles, although 4000 ppm was lethal to ladybird beetles. It is clear that the exposure of *A. gossypii* to Huwa-San for 48 h can produce serious and observable damage to the *A. gossypii* cuticle (Figure 3), thereby resulting in its death. The mode of action here is unknown and requires further investigations to clarify the site and biological basis of Huwa-San TR50 effects.

Huwa-San TR50 concentration of 2000 ppm did not cause malformation of cucumber leaves; however, beyond this concentration a significant leaf malformation was detected (Figure 2). This suggests that 2000 ppm is a safe concentration of Huwa-San TR50, for minimizing the effects on cucumber leaves and retaining the control of *A. gossypii.* This is because there was no statistically significant difference effect on the mortality of *A. gossypii* between 1000, 2000, 3000 and 4000 ppm. Moreover, 2000 ppm of Huwa-San TR50 had no significant effects on the mortality and behavior of both honeybees and ladybird beetles.

As mentioned previously, beneficial insects and natural enemies might be exposed directly or indirectly to insecticides. Hardstone and Scott (2010) reviewed the sensitivity of honeybees to six classes of insecticides (nicotinoids, carbamate, organophosphates, organochlorines, miscellaneous and pyrethroids) as compared with other insect pests. They pointed out that honeybees showed similar sensitivity to these six groups of insecticides as the targeted insect pests. This means that all available insecticides must be used completely to avoid exposing honeybees. Alaux et al. (2010) reported that honeybees exposed to imidacloprid can be significantly weakened resulting in increased mortality by *Nosemaceranae* infection, such that honeybees exposed to 1 ppb (part per billion) of fipronil and 5.1 ppm of thiacloprid showed an increased level of mortality in *N. ceranae* infection (Vidau et al., 2011). Previous laboratory studies have recorded that the learning and memory capacity of honeybees were affected upon exposure to a high concentration of neonicotinoids (Fairbrother et al., 2014).

Youn et al. (2003) highlighted that both acetamprid and imidacloprid were very toxic to Asian harlequin ladybird beetles (Pallas) (*Harmonia axyridis*), when applied at the recommended application rate for the control of aphids. In Bangladesh, Mollah et al. (2012) described the mortality of six synthetic chemical insecticides cypermethrin, fenitrothion, fenvalerate, emamectin benzoate, deltamethrin, esfenvalerate, curtap as well as the natural insecticide Neem oil on *C. septempunctata*.

In this study, honeybees and ladybird beetles were exposed directly to serial concentrations of Huwa-San TR50 up to 4000 ppm. They were also indirectly exposed to Huwa-San TR50 by feeding on either treated beeswax (a source of food for honeybees) or aphids (a source of food for ladybird beetles). Thus, both honeybees and ladybird beetles were exposed to Huwa-San TR50 by direct contact and indirectly by feeding. Both treatments had no significant observable effects on mortality and behaviors except for the high mortality of ladybird beetles at the highest concentration tested (4000 ppm). It is unclear why ladybird beetles were affected by 4000 ppm of Huwa-San TR50. However, in the field the potential of direct exposure of natural enemies and beneficial insects to insecticides is likely to involve indirect exposure at lower concentrations.

A possible limitation to the use of Huwa-San TR50 as a broad spectrum insect control agent is that it does not appear to produce any toxic effects on the larvae or adult male and female red palm weevil (*Rhynchophorus ferrugineus*) and no observable effects were detected as compared with the control up to 4000 ppm (unpubl result). It is likely that Huwa-San TR50 is much less toxic to tested insects belonging to the order Coleoptera (red palm weevil and ladybird beetles) and the order Hymenoptera (honeybees) whereas it is more toxic to the order Hemiptera (the cotton aphids) and this may reflect differences in the thickness or makeup of the cuticles of these different groups of insects.

5. Conclusion

Huwa-San TR50 seems to have the potential of controlling *A. gossypii* with low toxicity to both honeybees and ladybird beetles. This study has provided evidence showing that 2000 ppm of Huwa-San TR50 is responsible for 96.5% mortality of *A. gossypii* after 48 h of exposure and at this concentration there was no observable damage to cucumber leaves or beneficial insects.

Further investigations are required to address the following:

- The potential tree stress that might be produced by Huwa-San TR50.
- The toxicity of Huwa-San TR50 to soil microorganisms, and beneficial animals such as soil dwelling earthworms and nematodes.
- The best time and type of field application of Huwa-San TR50 should be determined, as well as any plant systemic properties.

Finally, the findings encourage us to test the effectiveness of Huwa-San TR50 on other types of aphids, as well as to screen the sensitivity of Huwa-San TR50 on other host plants of cotton aphids. Further studies will be necessary to identify its residues in food and the environment.

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